National Toxicology Program
Toxicity Report Series
Number 59

NTP Technical Report
on the Toxicity and Metabolism Studies of

Chloral Hydrate

(CAS No. 302-17-0)

Administered by Gavage
to F344/N Rats and B6C3F$_1$ Mice

Frederick A. Beland, Ph.D., Study Scientist
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U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals.

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This NTP report on the toxicity studies of chloral hydrate is based primarily on 16- and 17-day toxicity and metabolism studies that took place from June through October 1993 and the *in vitro* metabolism and DNA-binding studies that took place from April 1993 through December 1996.

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PEER REVIEW

The draft report on the toxicity and metabolism studies of chloral hydrate was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that the toxicity study report presents the experimental results and conclusions fully and clearly.

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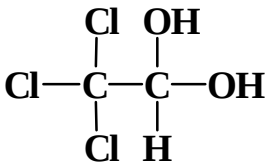
ABSTRACT



CHLORAL HYDRATE

CAS No. 302-17-0

Chemical Formula: $Cl_3CCH(OH)_2$ Molecular Weight: 165.40

Synonyms: 2,2,2-Trichloro-1,1-ethanediol; 1,1,1-trichloro-2,2-ethanediol; trichloroacetaldehyde monohydrate

Chloral hydrate is widely used as a sedative and a hypnotic in pediatric medicine. It is also a byproduct of water chlorination. Chloral hydrate has been shown to be genotoxic in numerous prokaryotic and eukaryotic assay systems including human lymphocytes *in vitro*. One of its metabolites, trichloroacetic acid, has demonstrated hepatocarcinogenic activity in mice. Trichloroethylene and perchloroethylene, both of which are metabolized to chloral hydrate, have been shown to be carcinogenic in rats and/or mice. Because of this evidence of carcinogenicity and because of the wide-spread use of chloral hydrate, 16- or 17-day range-finding toxicity studies and separate 16- or 17-day metabolism studies were performed in F344/N rats and B6C3F_1 mice in preparation for further long-term rodent studies. In addition, *in vitro* studies of the metabolism and DNA-binding capacity of chloral hydrate and its metabolites were performed. Genetic toxicity studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, *Drosophila melanogaster*, and mouse bone marrow cells.

For the range-finding studies, groups of eight male and eight female F344/N Nctr BR rats and B6C3F_1/Nctr BR (C57BL/6N×C3H/HeN MTV$^-$) mice were administered 0, 50, 100, 200, 400, or 800 mg chloral hydrate per kg body weight in water by gavage 5 days per week for 17 days (rats) or 16 days (mice) for a total of 12 doses. One male rat receiving 800 mg/kg died after five doses. Two 800 mg/kg female rats died after dosing ended but before study termination. One male mouse in each group except the 400 mg/kg group died before the end of the study. Two 800 mg/kg female mice also died before the end of the study. The final mean body weight of 800 mg/kg male rats and the mean body weight gains of 400 and 800 mg/kg males were significantly less than those of the vehicle controls. The mean body weight gains of all groups of dosed male mice were

significantly greater than that of the vehicle control group. The only clinical finding in rats and mice attributed to chloral hydrate treatment was light sedation in the 400 mg/kg groups and heavy sedation in the 800 mg/kg groups; sedation subsided within 30 minutes or 3 hours, respectively. The liver weights of 400 mg/kg male mice and 800 mg/kg male and female mice were significantly greater than those of the vehicle control groups. No chemical-related lesions were observed in rats or mice.

Male and female rats and mice were administered a single dose of 50 or 200 mg chloral hydrate per kg body weight in water by gavage, or 12 doses of 50 or 200 mg/kg over 17 days (rats) or 16 days (mice). Plasma concentrations of chloral hydrate and its metabolites were determined 15 minutes, 1, 3, 6, and 24 hours, and 2, 4, 8, and 16 days after receiving 1 or 12 doses. Maximum concentrations of chloral hydrate were observed at the initial sampling point of 15 minutes. By 1 hour, the concentrations had dropped substantially, and by 3 hours, chloral hydrate could not be detected in rats or mice. Trichloroacetic acid was the major metabolite detected in the plasma. In rats, the concentrations rose slowly, with the peaks occurring between 1 and 6 hours after treatment. In mice, the peak concentrations were found 1 hour after dosing. The concentrations then slowly decreased such that by 2 days the metabolite could no longer be detected in rats or mice. Trichloroethanol was assayed both as the free alcohol and its glucuronide. In rats, the maximum concentrations of free trichloroethanol occurred at 15 minutes, while the peak concentrations of trichloroethanol glucuronide were found at 1 hour; by 3 hours, concentrations of both metabolites approached background levels. In mice, the maximum concentrations of both metabolites occurred at 15 minutes, and by 1 to 3 hours concentrations approached background levels. The plasma concentrations of chloral hydrate and its metabolites were dose dependent in rats and mice. In mice, plasma concentrations of trichloroacetic acid were significantly higher after a single dose than after 12 doses. None of the metabolic parameters appears to account for species differences that may exist in hepatocarcinogenicity.

The data from the study of metabolism and DNA adduct formation indicated that *in vitro* metabolism of 200 μM to 5 mM chloral hydrate by male B6C3F$_1$ mouse liver microsomes (control microsomes) generated free radical intermediates that resulted in endogenous lipid peroxidation, forming malondialdehyde, formaldehyde, acetaldehyde, acetone, and propionaldehyde. Similar concentrations of trichloroacetic acid and trichloroethanol, the primary metabolites of chloral hydrate, also generated free radicals and induced lipid peroxidation. Lipid peroxidation induced by trichloroacetic acid nearly equaled that induced by chloral hydrate, while that from trichloroethanol was three- to fourfold less.

Metabolism of 200 μM to 5 mM chloral hydrate, trichloroacetic acid, and trichloroethanol by liver microsomes of B6C3F$_1$ mice pretreated with pyrazole (pyrazole-induced microsomes) yielded lipid peroxidation products

at concentrations two- to threefold greater than those from liver microsomes of untreated mice. Additionally, chloral hydrate-induced lipid peroxidation catalyzed by control and pyrazole-induced microsomes was reduced significantly by 2,4-dichloro-6-phenylphenoxyethylamine, a general cytochrome P_{450} inhibitor. Human lymphoblastoid transgenic cells expressing cytochrome P_{450}2E1 metabolized 200 to 5,000 μg/mL chloral hydrate to reactants inducing mutations, whereas the parental cell line was inactive.

The malondialdehyde-modified DNA adduct, 3-(2-deoxy-β-D-erythro-pentofuranosyl)pyrimido[1,2α]purin-10(3H)-one (MDA-MG-1), formed from the metabolism of 1 mM chloral hydrate, trichloroacetic acid, and trichloroethanol by control B6C3F_1 mouse liver microsomes, mouse pyrazole-induced microsomes, male F344/N rat liver microsomes, and human liver microsomes in the presence and absence of calf thymus DNA was also determined. When incubated in the absence of calf thymus DNA, the amount of malondialdehyde formed from metabolism by pyrazole-induced mouse microsomes was twice that from rat or human liver microsomes. Amounts of chloral hydrate-induced and trichloroacetic acid-induced lipid peroxidation products formed from metabolism by rat and human liver microsomes were similar, and these quantities were about twice those formed from the metabolism of trichloroethanol. The quantity of MDA-MG-1 formed from the metabolism of chloral hydrate, trichloroacetic acid, and trichloroethanol by mouse, rat, and human liver microsomes exhibited a linear correlation with the quantity of malondialdehyde formed under incubation conditions in the absence of calf thymus DNA.

Chloral hydrate was shown to be mutagenic *in vitro* and *in vivo*. At doses from 1,000 to 10,000 μg/plate, it induced mutations in *S. typhimurium* strain TA100, with and without S9 activation; an equivocal response was obtained in *S. typhimurium* strain TA98 in the absence of S9, and no mutagenicity was detected with strain TA1535 or TA1537. Chloral hydrate at doses from 1,700 to 5,000 μg/mL induced sister chromatid exchanges; at doses from 1,000 to 3,000 μg/mL, chromosomal aberrations were induced in cultured Chinese hamster ovary cells, with and without S9. Results of a sex-linked recessive lethal test in *D. melanogaster* were unclear; administration of chloral hydrate by feeding produced an inconclusive increase in recessive lethal mutations, results of the injection experiment were negative. An *in vivo* mouse bone marrow micronucleus test with chloral hydrate at doses from 125 to 500 mg/kg gave a positive dose trend.

In summary, due to the absence of chloral hydrate-induced histopathologic lesions in rats and mice, no-observed-adverse-effect levels (NOAELs) were based on body weights of rats and liver weights of mice. The NOAELs for rats and mice were 200 mg/kg. Chloral hydrate was rapidly metabolized by rats and mice, with trichloroacetic acid occurring as the major metabolite. Peak concentrations of trichloroacetic acid occurred more quickly in mice. Plasma concentrations of chloral hydrate were dose dependent, but metabolic rates were

unaffected by dose or sex. Chloral hydrate was mutagenic *in vitro* and *in vivo*. Metabolism of chloral hydrate and its metabolites produced free radicals that resulted in lipid peroxidation in liver microsomes of mice, rats, and humans. Induction of cytochrome P_{450}2E1 by pyrazole increased the concentrations of lipid peroxidation products; inhibition of cytochrome P_{450}2E1 by 2,4-dinitrophenylhydrazine reduced these concentrations. Metabolism of chloral hydrate and its metabolites by mouse, rat, and human liver microsomes formed malondialdehyde, and in the presence of calf thymus DNA formed the DNA adduct MDA-MG-1.